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REPORTS

Percutaneous Penetration of Methylglyoxal Bis(guanylhydrazone): Effects on Hairless Mouse Epidermis In Vivo*

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Topical methylglyoxal bis(guanylhydrazone) (MGBG) previously has been shown to produce partial clinical improvement in psoriasis. To enhance therapeutic activity, studies were undertaken to optimize MGBG percutaneous penetration in vitro and to study biochemical changes related to epidermal proliferation in vivo. MGBG penetration in saline, Vehicle N, decylmethylsulfoxide, and N-methylpyrrolidone was determined in normal human skin in vitro. Maximum penetration was obtained with 10% MGBG in Vehicle N (3 μg/h/cm²). Both topical and systemic MGBG resulted in increased levels of S-adenosyl-L-methionine decarboxylase, suggesting an extended half-life as a consequence of MGBG binding. Topical treatment with 10% MGBG in Vehicle N also resulted in decreased epidermal polyamine levels. The changes in polyamine metabolism were also associated with inhibition of epidermal DNA synthesis. These studies suggest that this topical MGBG formulation may be a candidate for use in the treatment of psoriasis and other hyperproliferative cutaneous diseases associated with increased polyamine synthesis.

The increased epidermal cell proliferation in psoriasis is associated with elevated tissue levels of the polyamines putrescine, spermine, and spermidine, as well as increased activities of the polyamine biosynthetic enzymes, ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (SAMDC) [1,2] (Fig 1). Various therapies effective in psoriasis, such as steroids, anthralin, PUVA, and etretinate all normalize polyamine abnormalities in parallel with clinical improvement [1,3,4]. These studies suggest that polyamines may play a role in the cellular regulatory factors in psoriasis and that modulation of polyamine metabolism by pharmacologic agents may therefore be useful for the treatment of this skin disorder.

In a recent clinical screen of topical chemotherapeutic agents, methylglyoxal bis(guanylhydrazone) (MGBG) a strong competitive inhibitor of SAMDC produced a partial therapeutic response in psoriasis ([5]. The topical formulation (10% MGBG in saline) used in this clinical trial was selected on an empirical basis without consideration for optimizing percutaneous penetration. The present study was therefore undertaken to evaluate the percutaneous penetration of MGBG in vitro to enhance penetration and to determine the effects of the selected MGBG formulations on epidermal DNA synthesis and polyamine metabolism in hairless mice in vivo.

MATERIALS AND METHODS

Methylglyoxal bis(guanylhydrazone) dihydrochloride monohydrate (MGBG) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin; Vehicle N (alcohol 47.5%, water, laureth-4, isopropyl alcohol 4%, propylene glycol) from Neutrogena Corp., Los Angeles, California; n-decylmethyl sulfoxide (C_{10}MSO) from Cyclo Chemical Co., Los Angeles, California; N-methylpyrrolidone from Nelson Research, Irvine, California. [Methyl-3H]thymidine (spec act 25.0 Ci/mmol) and S-[carboxyl-^{14}C]adenosyl-L-methionine (spec act 62.0 mCi/mmol) were purchased from Amersham, Arlington Heights, Illinois. TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) was purchased from Consolidated Midland Corporation, Brewster, New York.

In Vitro Percutaneous Penetration Studies

The percutaneous penetration of MGBG was measured in glass diffusion cells by the technique previously described [6]. MGBG (0.5 ml) in various vehicles was applied to the epidermal surface of excised normal human autopsy skin (0.2 ml/cm²). The dermal reservoir contained phosphate-buffered saline. Diffusion cells were incubated with constant stirring at 28°C. Three diffusion cells were run for each test condition. MGBG penetration was quantitated by high-performance liquid chromatographic (HPLC) analysis using the method previously described [7]. Samples of saline, Vehicle N, and C_{10}MSO without MGBG were chromatographed and found to contain no peaks that interfered with MGBG analysis. Standard curves for MGBG in saline were constructed and used for quantitation. The reproducibility and curve fit were excellent ($r_2 = 0.99$). Percutaneous samples were chromatographed without further purification.

The test solution was removed after the 48-h incubation period and the epidermal surface rinsed 3 times with the vehicle in which the drug was dissolved. This process effectively removed all surface drug that had not penetrated the stratum corneum. The epidermis was separated by heating the skin for 1 min in a 60°C water bath. To extract MGBG from the epidermis, tissues were homogenized in 1 ml of saline after which 100 μl of 10 N perchloric acid was added. This was mixed well and centrifuged at 10,000 *g* for 10 min. One hundred microliters of 10 N KOH was added to neutralize the supernatant which was then assayed for MGBG content by HPLC as described above.

In Vivo Studies

HRS J albino hairless mice aged 2–3 months were used for these experiments.

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Abbreviations:
C_{10}MSO: n-decylmethyl sulfoxide
DFMO: α-difluoromethyl ornithine
HPLC: high-performance liquid chromatography
MGBG: methylglyoxal bis(guanylhydrazone)
ODC: ornithine decarboxylase
SAM: S-[carboxyl-^{14}C]adenosyl-methionine
SAMDC: S-adenosyl-L-methionine decarboxylase
TPA: 12-*O*-tetradecanoyl-phorbol-13-acetate

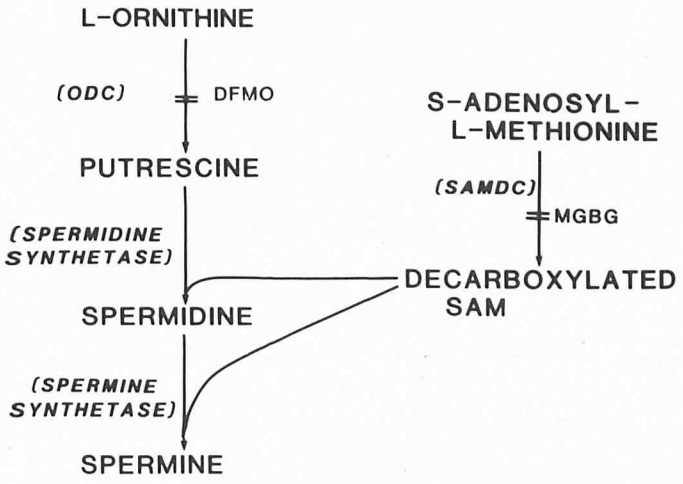

FIG 1. Polyamine biosynthetic pathway.

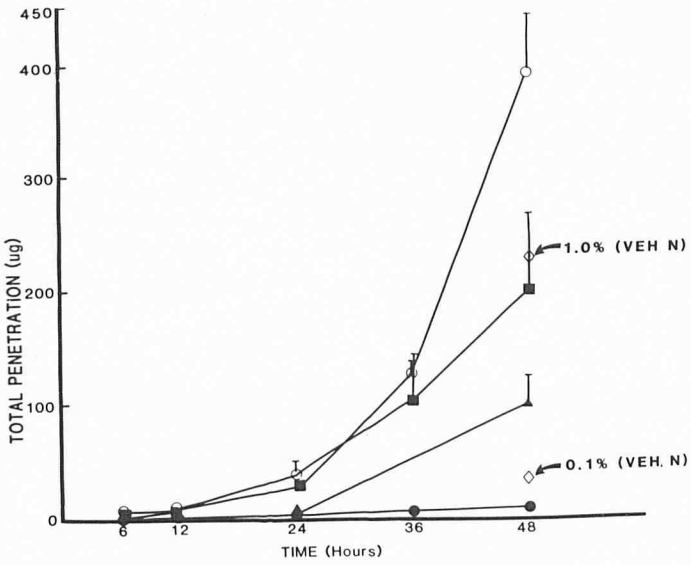

FIG 2. Effect of vehicle on 10% MGBG percutaneous penetration in vitro. Vehicles: ●, saline; ▲, N-methylpyrrolidone:isopropyl alcohol:water (43:30:27); ■, decylmethylsulfoxide:water (2.5:97.5); ○, Vehicle N. *Arrows* indicate 48-h penetration of 0.1% and 1.0% MGBG in Vehicle N. Mean values ± SD for 3 diffusion chambers.

1. *Effects of MGBG on DNA synthesis.* Fifty microliters of MGBG in Vehicle N or Vehicle N alone (control) was applied to the backs of normal hairless mice (6 mice/group) daily for either 3 or 7 days. Six hours after the last application mice were injected with tritiated thymidine (2 μCi/g body weight). Another group of animals was similarly treated except that TPA (17 nmol in a total volume of 0.2 ml acetone) was applied topically 12 h prior to the administration of tritiated thymidine. One hour after administration of isotope, animals were sacrificed by cervical dislocation and treated skin excised. Epidermis was removed after incubating the skin specimens for 1 h at 37°C in 2 M sodium bromide. DNA was extracted and assayed using the technique described by Halprin et al [8]. Results of epidermal DNA synthesis were calculated as cpm per μg DNA. Another group of 6 mice received systemic (i.p.) MGBG (50 mg/kg) at 12 and 6 h prior to the administration of tritiated thymidine. Skin biopsies were also processed for standard histology.

2. *Effects on polyamine biosynthesis.* Fifty microliters MGBG/Vehicle N or Vehicle N (control) was applied daily for either 3 or 7 days or given i.p. (50 mg/kg) at 6 and 12 h prior to sacrifice (8 mice/group). TPA (17 nmol) was applied 12 h before sacrifice to stimulate epidermal SAMDC activity [9]. The dorsal skin samples were pooled from 4 animals to determine SAMDC activity. SAMDC levels were determined in epidermal homogenates by the release of $^{14}CO_2$ from S-[carboxyl-^{14}C]adenosyl-methionine (SAM) as previously described [10].

For determining MGBG effects on polyamine levels, mice were treated as described above (8 mice/treatment group) and dorsal skin samples from 2 mice were pooled for polyamine analysis. Epidermal polyamine levels were quantitated by an amino acid analyzer using the technique previously described [11].

RESULTS

In Vitro Percutaneous Penetration Studies

The percutaneous penetration of 10% MGBG was determined in saline and 3 other vehicles that have been reported to enhance penetration of various drugs. There was an increase in penetration at 24 and 48 h in C_{10}MSO and Vehicle N compared to saline (Fig 2). Vehicle N produced the maximum rate of MGBG penetration of 3 μg/h/cm^2 surface area of skin. There was a dose-dependent increase in MGBG penetration in

TABLE I. *In vitro percutaneous penetration study—epidermal MGBG content*

Vehicle	MGBG content of epidermis at 48 h (μg (mean ± SD))
Vehicle N	525 ± 81
C_{10}MSO:H_2O (2.5:97.5)	1191 ± 266
Saline	58 ± 25
N-Methylpyrrolidone:isopropyl alcohol:H_2O (43:30:27)	185 ± 85

Ten percent MGBG in the above vehicles was applied to the epidermal surface of human skin in vitro. Upon completion of the study at 48 h (Fig 2), epidermis was removed and MGBG content assayed as described in *Materials and Methods.*

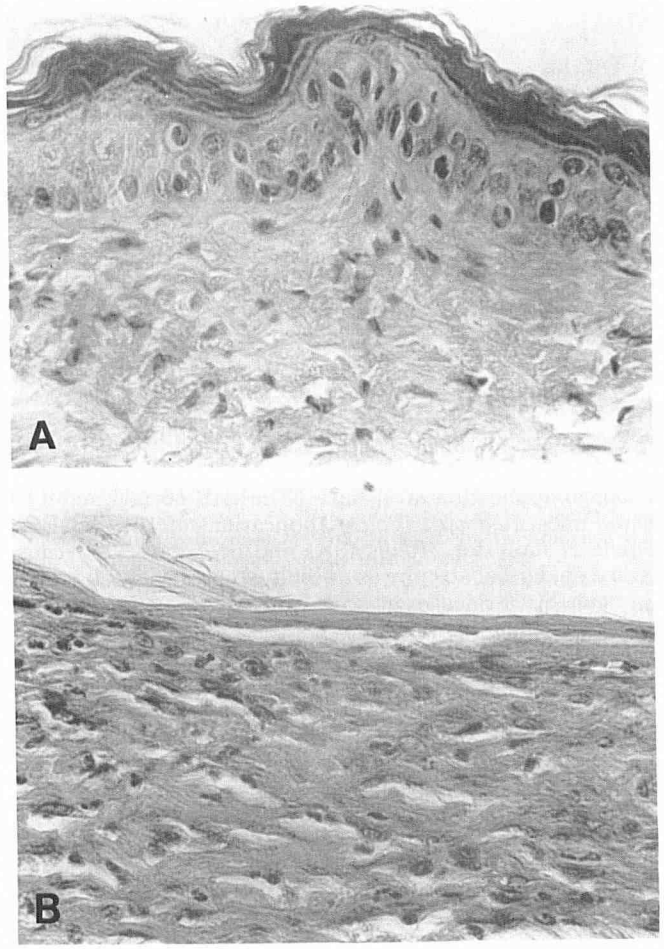

FIG 3. Effects of topical MGBG in Vehicle N on normal mouse skin applied daily ×7. *A*, Vehicle N control. *B*, Ten percent MGBG. × 400.

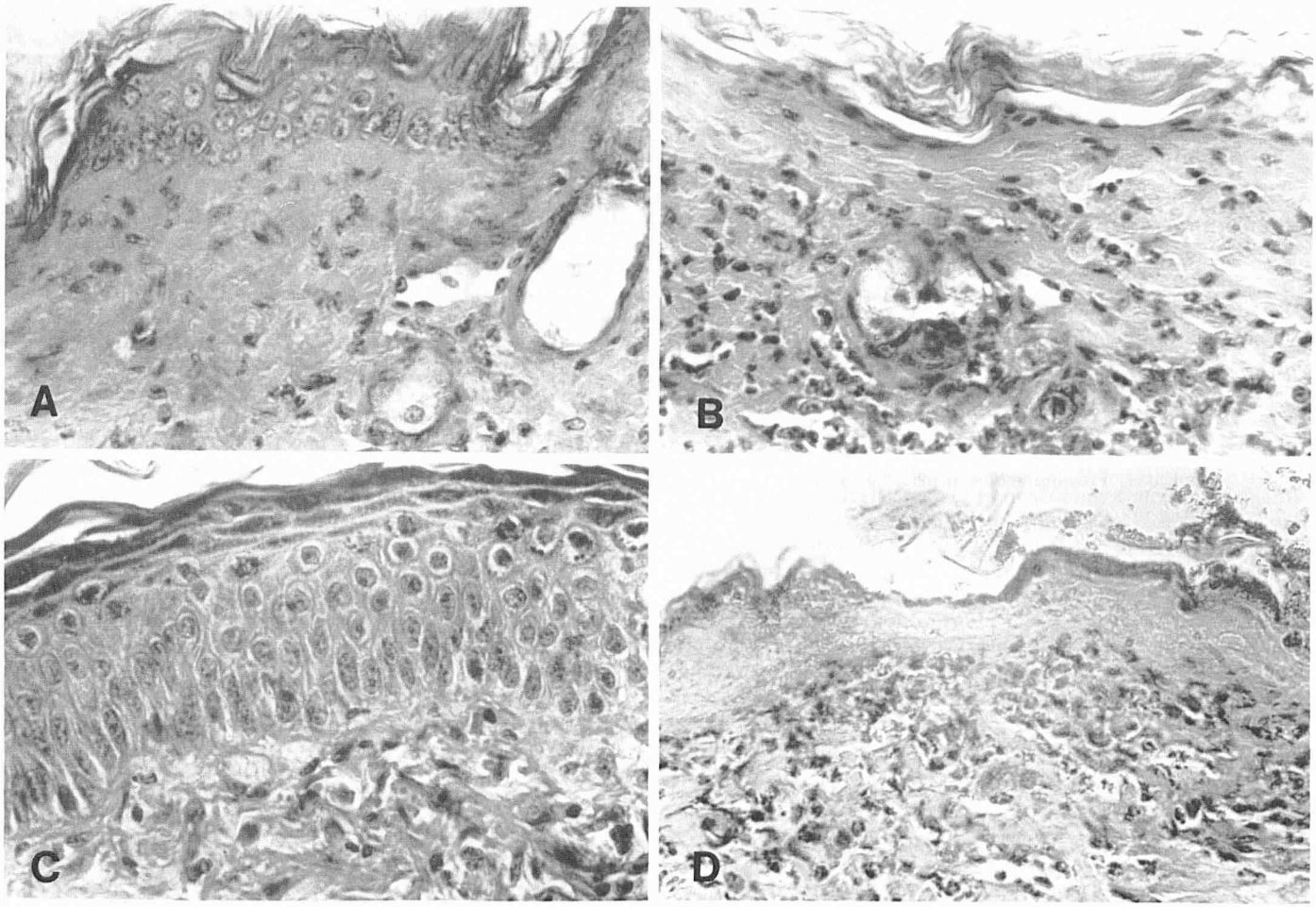

FIG 4. Effects of topical MGBG in Vehicle N on TPA-treated mouse skin, applied daily ×3; *A*, Vehicle N control. *B*, Ten percent MGBG; or applied daily ×7. *C*, Vehicle N control. *D*, Ten percent MGBF. × 400.

Vehicle N comparing 0.1%, 1%, and 10% MGBG penetration at 48 h (Fig 2). The penetration of 0.1% MGBG in Vehicle N exceeded that of 10% MGBG in saline. Vehicle N and C_{10}MSO vehicles also produced a significant increase in MGBG epidermal content compared to saline and N-methylpyrrolidone vehicles (Table I).

In Vivo Studies

Epidermal hyperplasia characterized by acanthosis (Figs 3*A*, 4*A*,*C*) and increased DNA synthesis (Table II) was produced by topical application of Vehicle N in both normal and TPA-treated mice. Repeated topical applications of 10% MGBG in Vehicle N daily for 3 days (3×) and/or 7 days (7×) caused marked epidermal atrophy as a result of epidermal cell destruction seen histologically in both normal (Fig 3*B*) and TPA-stimulated epidermis (Fig 4*B*,*D*).

Topical MGBG (×3) or systemic MGBG had no effect on epidermal DNA synthesis in normal mouse skin, whereas topical MGBG (×7) produced 50% inhibition of DNA synthesis (Table II). In TPA-stimulated epidermis, topical MGBG (×3) and (×7) produced 41% and 81% inhibition of epidermal DNA synthesis, respectively (Table II). The low levels of SAMDC in normal hairless mouse epidermis are induced 6- to 7-fold between 9–12 h following a single application of 17 nmol TPA [10]. In the present study, the topical application of MGBG (×3) and (×7) resulted in increased SAMDC levels compared to vehicle controls in TPA-stimulated epidermis (Table III). The hyperproliferation produced by the 7 daily applications of Vehicle N resulted in a 10-fold increase in SAMDC compared to the TPA control. Systemic MGBG produced an enhancement of SAMDC activity similar to that produced by the topical (×3) application.

TABLE II. *Effect of topical and systemic MGBG on epidermal DNA synthesis*

	CPM/μG DNA	% Inhibition
Normal mice		
Vehicle N control (×3)	5.3 ± 2.2	—
Topical MGBG (×3)	7.0 ± 2.0	–0–
Vehicle N control (×7)	10.7 ± 1.6	—
Topical MGBG (×7)	5.4 ± 0.6	50 ($p < 0.0005$)
Untreated control	3.4 ± 0.4	—
Systemic MGBG	4.3 ± 1.7	–0–
TPA-treated mice		
Vehicle N control (×3)	25.5 ± 4.8	—
Topical MGBG (×3)	15.0 ± 3.5	41 ($p < 0.025$)
Vehicle N control (×7)	17.1 ± 4.8	—
Topical MGBG (×7)	3.9 ± 1.2	86 ($p < 0.0005$)

Topical applications of MGBG (10%) in Vehicle N, or Vehicle N (control) and systemic MGBG administration (50 mg/kg × 2) to normal and TPA-treated mice is described in *Materials and Methods*. Each treatment group consisted of 6 mice.

In TPA-treated mice topical MGBG (×3) produced a 2-fold increase in putrescine (control = 17 ± 3 nmol/mg protein), with a 30% decrease in spermidine (control = 86 ± 1 nmol/mg protein) and no change in spermine levels (control = 8 ± 1 nmol/mg protein) (Fig 5). After 7 days of treatment (Vehicle N × 7 controls: putrescine, 27 ± 4 nmol/mg protein; spermidine,

TABLE III. *Effect of topical and systemic MGBG on TPA-stimulated epidermal SAMDC*

Treatment	SAMDC activity (pmol/CO_2 released/h/mg protein)
Vehicle N (×3)	17 ± 4
Topical MGBG (×3)	118 ± 45
Vehicle N (×7)	605 ± 247
Topical MGBG (×7)	1460 ± 273
Control	18 ± 8
Systemic MGBG	130 ± 45

Animals were treated with topical applications of MGBG (10%) in Vehicle N, or Vehicle N (control) daily for 3 or 7 days or MGBG was administered systemically (50 mg/kg) at 6 and 12 h prior to sacrifice. All animals received topical application of TPA (17 nmol) 12 h prior to sacrifice to stimulate SAMDC.

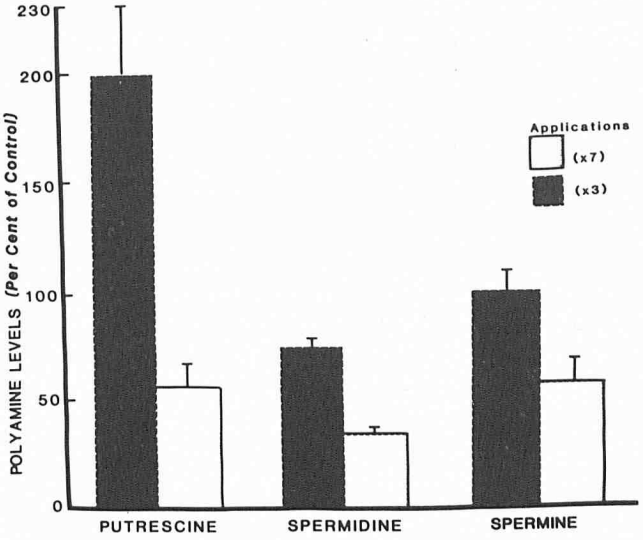

FIG 5. Effects of topical 10% MGBG in Vehicle N applied daily ×3 or daily ×7 on epidermal polyamine levels in TPA-treated mice. Results are expressed as percent of Vehicle N treated control (mean ± SD).

65 ± 2 nmol/mg protein; spermine, 7 ± 1 nmol/mg protein), the putrescine and spermine levels dropped to half of the controls, with a 70% decrease in spermidine.

DISCUSSION

The elevation of epidermal polyamine levels in psoriasis and their normalization by various effective treatment modalities provides a basis for the potential therapeutic use of inhibitors of polyamine biosynthesis. MGBG, a competitive inhibitor of SAMDC, is a potent antiproliferative agent used in the treatment of malignant disease. The substantial cytotoxic side effects of MGBG preclude its systemic use in a benign disease such as psoriasis. Topical application of MGBG (10% in saline) has been shown to produce a partial therapeutic response in psoriasis [5]. In the present investigation we have shown that Vehicle N provides enhanced topical delivery of MGBG, with penetration of 0.1% MGBG in Vehicle N exceeding that of 10% in saline.

The rapid rate of synthesis of the target enzyme, SAMDC, requires the continued presence of high concentrations of MGBG to maintain an inhibitory effect in vivo [12]. The increased penetration of MGBG in Vehicle N and the sustained release achieved by the topical route may optimize the therapeutic activity of MGBG.

The effects of topical MGBG on epidermal polyamines and their biosynthetic enzymes were entirely as predicted from other systems [12]. The increase in epidermal SAMDC levels suggests an extended half-life or stabilization as a consequence of MGBG binding. The functional inhibition of SAMDC is supported by the initial increase in putrescine levels. The minimal effect on spermidine and no effect on spermine after 3 daily applications is consistent with the slower rates of degradation of these polyamines once they have been synthesized in vivo. The decrease in all polyamine levels after treatment for 7 days may reflect the extensive epidermal destruction as seen histologically.

The decrease in polyamine levels in TPA-treated hyperproliferative epidermis is associated with an inhibition of DNA synthesis resulting in an atrophic epidermis presumably from a marked decrease in cell proliferation. In view of the substantial increase in percutaneous penetration of this topical MGBG formulation and its biochemical effects on hyperproliferative epidermis, this preparation may be useful for the treatment of psoriasis and other hyperproliferative cutaneous diseases associated with increased polyamine synthesis.

Topical treatment with α-difluoromethyl ornithine (DFMO), an irreversible inhibitor of ODC (Fig 1) has recently been shown to produce moderate clinical improvement in psoriasis [13]. Kousa et al [14] have shown that the intradermal administration of MGBG or DFMO causes a depletion of polyamine levels in psoriasis. The "priming" effect of DFMO to enhance epidermal accumulation of MGBG has recently been demonstrated in mouse skin [15,16]. Further research directed toward the combination of topical DMFO and MGBG may lead to a more effective and less toxic therapeutic regimen for the treatment of psoriasis and other hyperproliferative cutaneous disorders.

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Diagnostic Criteria in Sézary's Syndrome: A Multiparameter Study of Peripheral Blood Lymphocytes in 32 Patients with Erythroderma

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In order to define additional diagnostic criteria for the early diagnosis of Sézary's syndrome (SS), peripheral blood lymphocytes of 32 patients with erythroderma, including 8 patients with SS, 4 patients with erythrodermic mycosis fungoides, 14 patients with an erythroderma on the basis of atopic or chronic dermatitis, and 6 patients with erythrodermic psoriasis, were investigated by computer-assisted morphometry. The degree of nuclear indentation, expressed as the nuclear contour index (NCI), was measured on electron micrographs. The mean NCI and the percentages of cerebriform mononuclear cells (CMC), defined by a NCI ≥ 6.5, were calculated. In addition, the percentages of lymphocytes, T and B cells, and the distribution of T-cell subpopulations as defined by Fc-receptors (Tμ, Tγ) and monoclonal antibodies (OKT3, OKT4, OKT8, HLA-DR) were determined.

Statistical analysis showed as most discriminating parameters for the differentiation between SS and benign forms of erythroderma: high percentages of lymphocytes (50% or more), an expanded $OKT3^+$, $OKT4^+$ population with an OKT4/OKT8 ratio > 10, a mean NCI value ≥ 5.5, the presence of more than 20% CMC, as well as the presence of cells with a NCI ≥ 11.5. The total leukocyte and lymphocyte counts, as well as the percentages of B, T, Tμ, and Tγ cells had limited value for the early diagnosis of SS.

Sézary's syndrome (SS), first described in 1938 [1] is clinically characterized by a pruritic exfoliative or infiltrated erythroderma, lymphadenopathy, and the presence of atypical mononuclear cells (Sézary cells) in the peripheral blood [2,3]. At present most investigators regard SS as a neoplastic disease and part of the spectrum of cutaneous T-cell lymphomas (CTCL) [4,5]. In the early stage of the disease the clinical and histologic features are not diagnostic and differentiation from erythroderma on the basis of chronic dermatitis, atopic dermatitis, or psoriasis is difficult. In these patients with generally normal leukocyte counts the diagnosis rests mainly upon demonstration of Sézary cells in the peripheral blood. However, objective criteria for recognition of Sézary cells by light microscopy are lacking [6]. Moreover, ultrastructural studies have demonstrated the presence of cerebriform mononuclear cells (CMC), similar to those in SS, in the peripheral blood of patients with generalized benign dermatoses [7].

In order to define additional diagnostic criteria for the early diagnosis of SS, we have performed computer-assisted morphometry and marker studies of peripheral blood lymphocytes of 32 patients with erythroderma, including 8 patients with SS, 4 with erythrodermic mycosis fungoides (MF), and 20 patients with various benign forms of erythroderma.

MATERIALS AND METHODS

Patients

Heparinized venous blood was obtained from 32 patients with erythroderma including 8 patients with SS, 4 with erythrodermic MF, 14 with chronic dermatitis, and 6 with psoriasis. The peripheral blood from 10 healthy donors served as controls.

The diagnosis of SS was based on clinical and histologic criteria: erythroderma, lymphadenopathy, and the presence of hyperconvoluted lymphoid cells, compatible with Sézary cells, in the peripheral blood.

The patients with erythrodermic MF had shown the classical Alibert-Bazin type of MF for 3 months (see Table I, no. 9) to more than 4 years (nos. 10–12) before the erythroderma developed. At the time of study all patients had enlarged inguinal lymph nodes. Histologic examination showed dermatopathic lymphadenopathy in 2 patients (nos. 9 and 10), early involvement according to the criteria of Scheffer et al [8] in 1 patient (no. 11), and diffuse MF involvement in another patient (no. 12) with progressive leukemic disease, which proved to be fatal within 5 months. The clinical data of the patients with SS and MF at the time of study are shown in Table I.

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Abbreviations:
CMC: cerebriform mononuclear cells
CTCL: cutaneous T-cell lymphoma
FCS: fetal calf serum
MF: mycosis fungoides
NCI: nuclear contour index
PBS: phosphate-buffered saline
SS: Sézary's syndrome